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High performance liquid chromatographic separation of interesterified palm oil with tributyrin

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Abstract

Short-chain triacylglycerol, tributyrin (glyceryl-*sn*-1,2,3-tri-butyric acids), was interesterified with palm oil to produce mixtures of structured triacylglycerol (SL-TAG) species as a low-calorie lipid. Lipozyme RM IM from *Rhizomucor miehei*, known as a 1,3-regioselective immobilized lipase, was used as a catalyst. During 24 h reaction, the reaction mixture was analyzed with Hypersil® BDSCPS high performance liquid chromatography (HPLC) column to determine neutral lipids composition. Also, the compositional changes of TAG, as well as monoacylglycerol (MAG)/diacylglycerol (DAG) as by-products, were determined. After 24 h, 5.8 g/100 g DAG was found in the reaction mixture, while 89.5 g/100 g TAG was observed. The reaction mixture was also analyzed with Nova-Pak® C18 and Chrompack Si60 columns for separation of SL-TAG species. It was found that the normal-phase (NP)-HPLC with Chrompack Si60 column readily separated SL-TAG species composed of short- and long-chain acyl residues. Further, mass spectrometer was employed for identifying the separated SL-TAG molecules. SL-TAG containing two butyryl and one long-chain acyl residue (e.g., PBB and OBB) were most abundant in the interesterified product.

Keywords: APCI mass spectrometry; Evaporated light-scattering detection; High performance liquid chromatography; Interesterification; Palm oil; Tributyrin

1. Introduction

Palm oil is one of the traditional fats is human diet and has been widely used throughout the world. In 2005 global palm oil production was 33,733,000 metric tones, accounting for 24% of the worldwide production of total dietary oil (Basiron, 2007). Recently, compositional modification of palm oil is an important application, in which functional fatty acids (i.e., conjugated fatty acid, medium- or short-chain fatty acid, omega-3 fatty acid) are incorporated into triacylglycerol (TAG) molecules in palm oil.

Structured lipids (SL) are TAG molecules that have intentionally predetermined fatty acyl groups on the glycerol backbone. Through lipase-catalyzed interesterification, for example, such modified fats/oils can be obtained by exchanging acyl groups between ester bondages of lipids, resulting in improved characteristics due to the change in composition and distribution of fatty acids (Akoh, 1995; Akoh & Moussata, 2001; Iwasaki & Yamane, 2000; Lee & Akoh, 1998; Senanayake & Shahidi, 2002; Torres, Munir, Blanco, Otero, & Hill, 2002; Willis, Lencki, & Marangoni, 1998). However, during interesterification between TAG molecules, mono- (MAG) and diacylglycerol (DAG) are produced as by-products along with the newly synthesized SL-TAG.

Use of SL as a low-calorie lipid is of interest. SL with saturated short-chain fatty acids can be intentionally produced because they provide about 3.5–6 kcal/g whereas long-chain fatty acids provide about 9–9.5 kcal/g. SALATRIM™ (*short and long acyltriglyceride molecules*) is one example of low-calorie lipid, produced by interesterification

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of highly hydrogenated vegetable oils with TAGs of short-chain fatty acids (acetic, propionic, and/or butyric acids) (Huang et al., 1994; Smith, Finley, & Leveille, 1994; Softly et al., 1994). Recently, separation of SL on reverse or normal-phase (NP) high-performance liquid chromatography (HPLC) and detection with evaporated light-scattering detector (ELSD) have been extensively studied (Lee, Jones, Lee, Kim, & Foglia, 2003; Ruiz-Gutiérrez & Barron, 1995) because the ELSD is not very sensitive to the change in mobile phase gradient during separation. Otherwise, refractive index detector is recommended for isocratic separation while ultraviolet detector has limitation on the detection of lipid species that lack double bonds. Generally, HPLC is suitable for the separation of TAG molecules while analysis of TAG containing short-chain fatty acids using NP- and reverse-phase (RP)-HPLC has not been widely applied. Mangos, Jones, and Foglia (1999) reported the NP-HPLC separation of TAG molecules containing acetic and long-chain fatty acids in which interesterification of triacetin and hydrogenated soybean oil was conducted.

In this study, an immobilized *Rhizomucor miehei* lipase (Lipozyme RM IM) was used for interesterification of palm oil with tributyrin to produce SL as a low-calorie lipid. During 24 h reaction, compositional changes of TAG as well as MAG/DAG as by-products were studied. The compositional changes of SL-TAG molecular species were examined using RP-HPLC. Finally, the reaction mixture was separated by NP-HPLC with mass spectrometric detection (MSD) to characterize the individual SL-TAG molecular species in SL products.

2. Material and methods

2.1. Materials

Tributyrin (glyceryl-*sn*-1,2,3-tri-butyric acids), tripalmitin (glyceryl-*sn*-1,2,3-tri-palmitic acids), tristearin (glyceryl-*sn*-1,2,3-tri-stearic acids), triolein (glyceryl-*sn*-1,2,3-tri-oleic acids), and trilinolein (glyceryl-*sn*-1,2,3-tri-linoleic acids) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Palm oil was a gift from CJ Co. (Seoul, Korea). The fatty acids composition of palm oil (g/100 g) are C16:0, 44.7; C18:0, 5.4; C18:1, 39.8, and C18:2, 10.2. All solvents were HPLC grade. Hexane, acetone, acetonitrile and ethylene chloride were obtained from Burdick & Jackson (Muskegon, MI, USA). Methyl-*t*-butyl ether (MTBE) was obtained from J.T. Baker Inc. (Phillipsburg, NJ, USA). Immobilized lipase (Lipozyme RM IM) was provided by Novo Nordisk Biochem North America Inc. (Franklinton, NC, USA).

2.2. Procedures for obtaining interesterified lipids

Tributyrin (0.5 g) was interesterified with an equal weight of palm oil using RM IM lipase (0.1 g, 10 wt% of substrates). Reactions were performed for 24 h at 65 °C. A screw-cap vial placed in a water-jacketed beaker with magnetic stirring was used as a reactor. During 24 h interesterification, reaction mixture was obtained at the designated reaction time (1, 3, 5, 7, 14, and 24 h) and was diluted with 100 mL hexane. Then, aliquot (3 mL) was filtered through a disposable Fluoropore PTFE membrane filter (Sigma Chemical). After evaporating hexane under nitrogen, each sample was re-dissolved in hexane (for normal phase) or acetone (for reverse phase) for HPLC analysis (Lee, Jones, & Foglia, 2002). As standards for HPLC, 0.5 g each of tripalmitin, tristearin, triolein, and trilinolein were, respectively, interesterified with an equal weight of tributyrin under the reaction conditions described above.

2.3. Analysis of interesterified lipids by HPLC

For separation of TAG, DAG, and MAG, a Hypersil® BDSCPS column (4.6 mm × 250 mm, 5 μm, Thermo Electron Corp., UK) was used in the HPLC system, which includes Younglin SP 930D dual pump (Younglin, Anyang, Korea). Sedex 75 ELSD (Afortvill, France) was operated at 40° with nitrogen as a nebulizing gas at a pressure of 2.2 bar. A binary solvent system of hexane and methyl-*t*-butyl ether with each solvent fortified with 0.4 g/100 g acetic acid was used. The flow rate was 1 mL/min. Hexane (100%) was pumped for 5 min, followed by an isocratic period of 10 min, then methyl-*t*-butyl ether (0–80%, v/v) was increased, linearly for 7 min, followed by increase of hexane to 100% for 0.1 min, and an isocratic period of 9.9 min. The area of each peak was integrated by Autochro-2000 software (Younglin).

Non-aqueous RP-HPLC was conducted on the 24-h interesterified reaction mixture with a Nova-Pak® C18 60 Å 4 μm HPLC column, (3.9 mm × 150 mm, Waters Corp., USA). The HPLC system was Younglin SP 930D dual pump (Younglin). Sedex 75 evaporative light-scattering detector (ELSD, Alfortvill, France) was operated at 30 °C with nitrogen as a nebulizing gas at a pressure of 1.5 bar. A binary step gradient of ethylene chloride in acetone was used at a flow rate of 0.8 mL/min using a solvent elution profile: linear gradient from 0% to 15% ethylene chloride (v/v) for 10 min; followed by a linear increase from 15% to 45% ethylene chloride (v/v) over 10 min; hold for 10 min; followed by a linear increase from 45% to 60% ethylene chloride (v/v) over 5 min; and hold for 5 min.

HPLC-mass spectrometry (MSD) was performed with a Hewlett-Packard Model 1050 HPLC coupled with a HP Model 5989A quadrupole mass spectrometer engine interfaced with the Analytica APCI attachment (HP Model 103722) in the positive ion mode. Rheodyne 7125 mannual injector (Cotati, CA, USA) with a10 μL loop was used. The separation was performed with a Chrompack Si60 column (300 × 3.0 mm i.d., 5 μm, Varian Instruments, Walnut Creek, CA, USA) at a solvent flow rate of 0.43 mL/min. A binary solvent system of 15% methyl-*t*-butyl ether and 85% hexane (each solvent was fortified with 0.4% acetic acid) was used as mobile phase using the separation conditions described previously (Lee et al., 2003). Nitrogen was used as drying gas (temperature, 350 °C) and nebulizing gas (temperature, 350 °C; pressure, 50 psi). Mass spectrometer parameters were as follows: EM voltage, −2906; HED voltage, -10^4; CapEx, 100; and quadrupole temperature, 150 °C. Full mass spectra were recorded every 0.7 s. To obtain area% of each separated peak, the products were analyzed with Hewlett-Packard Model 1050 HPLC equipped with a Varex MKIII ELSD (Burtonville, MD, USA). The operation temperature of the ELSD was 30 °C and nitrogen was the nebulizing gas at a pressure of 1.5 bar.

3. Results and discussion

During interesterification with RM IM lipase, palm oil, and tributyrin as TAG molecules are initially hydrolyzed, producing partial acylglycerols such as DAG and MAG. Equivalent amounts of FFA that was hydrolyzed from TAG molecules were also generated, simultaneously. Then subsequent rearrangements of the partial acylglycerols and FFA molecules occur to form a complex mixture of SL-TAG molecular species, having combinations of butyric acids with long-chain fatty acids originally from palm oil. Hence, after the reaction, SL-TAG with one butyryl, SL-TAG with two butyryl residues, DAG, MAG, and FFA would be present in the reaction mixture along with palm oil and tributyrin that did not participate in the reaction. Fig. 1 shows the complex mixture of SL-TAG molecular species after interesterification of palm oil with tributyrin.

Fig. 2 shows the HPLC separation of neutral lipid species after 1 h interesterification by Hypersil® BDSCPS column, in which FFA, MAG, DAG, and TAG were separated. During 24 h interesterification, reaction mixture was sampled at the designated reaction time (1, 3, 5, 7, 14, and 24 h), and the compositional changes of neutral lipid species (TAG, DAG, MAG, and FFA) were determined (Table 1). Before reaction, TAG was predominant in the reaction mixture (99.8 g/100 g). The amount of DAG was increased at prolonged reaction times, resulting in the reduction of TAG. After 24 h, 5.8 g/100 g DAG was found in the reaction mixture while 89.5 g/100 g TAG was observed, showing partial acylglycerols such as DAG and MAG were present in the reaction mixture. After 1 h reaction, TAG accounts for 96.5 g/100 g, and later this content decreased up to 89.5 g/100 g of the reaction mixture after 24 h (Table 1). Within 24 h reaction, sum of contents from MAG and DAG gradually increased up to 7.1 g/100 g, indicating that SL-TAG species along with MAG and DAG were produced as by-products.

Generally, RP-HPLC requires organic solvents such as acetone in conjunction with polar solvents for separation of TAG molecules. However, RP-HPLC has certain limitation in analyzing TAG because TAG molecules containing long-saturated fatty acids have poor solubility. In spite of such limitation, RP-HPLC can be successfully applied for separation of TAG-containing long- and

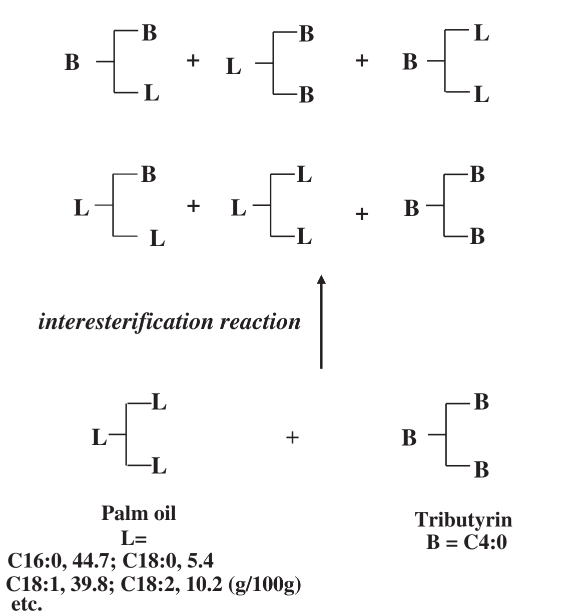

Fig. 1. Expected triacylglycerol structures produced in the interesterification of palm oil with tributyrin. (L, long-chain fatty acyl moiety and B, butyryl acyl moiety).

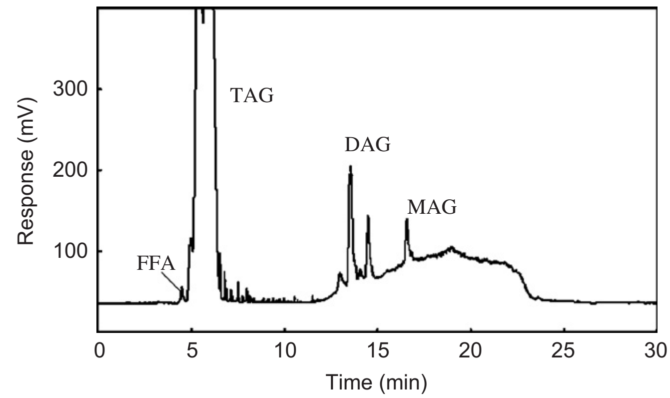

Fig. 2. Chromatogram of free fatty acid (FFA), monoacylglycerol (MAG), diacylglycerol (DAG), and triacylglycerol (TAG) in the interesterified product from 1 h reaction.

Table 1
The content (g/100 g) of mono-acylglycerol (MAG), di-acylglycerol (DAG), and tri-acylglycerol (TAG) in the interesterified products by normal-phase HPLC during 24 h

Reaction time (h)	FFA	MAG	DAG	TAG
0	–	–	0.2±0.1	99.8±0.5
1	0.3±0.1[a]	0.7±0.1	2.5±0.1	96.5±1.7
3	0.8±0.1	0.9±0.1	3.2±0.2	95.1±1.5
5	2.1±0.1	1.1±0.1	4.5±0.4	92.3±1.6
7	2.8±0.1	0.9±0.1	4.6±0.3	91.7±1.2
14	3.0±0.1	1.2±0.1	5.1±0.3	90.7±0.8
24	3.3±0.2	1.3±0.1	5.8±0.3	89.5±0.8

[a]Means and standard deviation of duplicates.

medium-saturated fatty acids (Foglia, Petruso, & Feairheller, 1993; Lee et al., 2002). Therefore, we modified our previously reported RP-HPLC methods that were developed for separation of long- and medium-chain TAG and applied it to the analysis of SL-TAG molecules from the interesterification of palm oil and tributyrin.

The separation by RP-HPLC is based on the combination of their theoretical carbon number and number of double bonds. Fig. 3 shows the RP-HPLC chromatograms after 0 h (Fig. 3A), 3 h (Fig. 3B), and 24 h reactions (Fig. 3C) of tributyrin and palm oil. After separation, the complete identification of SL-TAG species appeared complicated. All chromatograms were divided into two segments based on the peaks from palm oil and tributyrin before reaction, where each segment showed several peaks (not separated clearly) because lipid molecules in this analysis condition would tend to elute very close together in a given area of the chromatogram. In the chromatograms, SEG 2 was considered as palm oil that was not completely used during the reaction. Meanwhile, new peaks appeared in SEG 1, and these peaks were assumed to be SL-TAG molecules after interesterification of palm oil and tributyrin. In general, TAG molecules that have two short-chain acyl residues are more polar than TAG containing one short-chain fatty acyl residue, which results in their earlier elution in RP-HPLC. Therefore, it is plausible that peaks in SEG 1 of Fig. 3C represent SL-TAG molecules that have one long chain and two butyryl residues, along with possible DAG molecules (as by-products in the reaction) in region-I while region-II contained SL-TAG molecules that have two long chain and one butyryl residues. Such separation pattern was observed previously (Lee et al., 2002). Within 24 h reaction, areas of region-I and -II in SEG 1 indicated that SL-TAG species with two butyryl residues are more abundant than SL-TAG with one butyryl residue. This supposition was substantiated by the separation with NP-HPLC.

The chromatogram of the normal-phase separation of SL-TAG product obtained at 24 h interesterification is presented in Fig. 4. As discussed above, the interesterification of palm oil with tributyrin was expected to produce a mixture of SL-TAG molecules composed of mixed short- and long-chain acyl moieties. Theoretically, BBL/LBB [*sn*-1,2-butyl-3-long/*sn*-1-long-2,3-butyl] and LBL [*sn*-1-long-2-butyl-3-long] SL-TAG molecules could be synthesized where B is butyroyl moiety from tributyrin and L is long-chain fatty acyl moiety from palm oil (Fig. 1). The lipase from *R. miehei*, which was used in this reaction, is regarded as *sn*-1,3 selective on TAG. Therefore, the lipase partially hydrolyzed palm oil and tributyrin to form DAG and MAG, leaving liberated FFA in the initial stages of the reaction. Because palm oil contains palmitic (44.7 g/100 g), oleic (39.8 g/100 g), linoleic (10.2 g/100 g), and stearic acid (5.4 g/100 g) as major fatty acids, the DAG and MAG from palm oil are also composed of several combinations of such long-chain fatty acids (mostly palmitic and oleic acid).

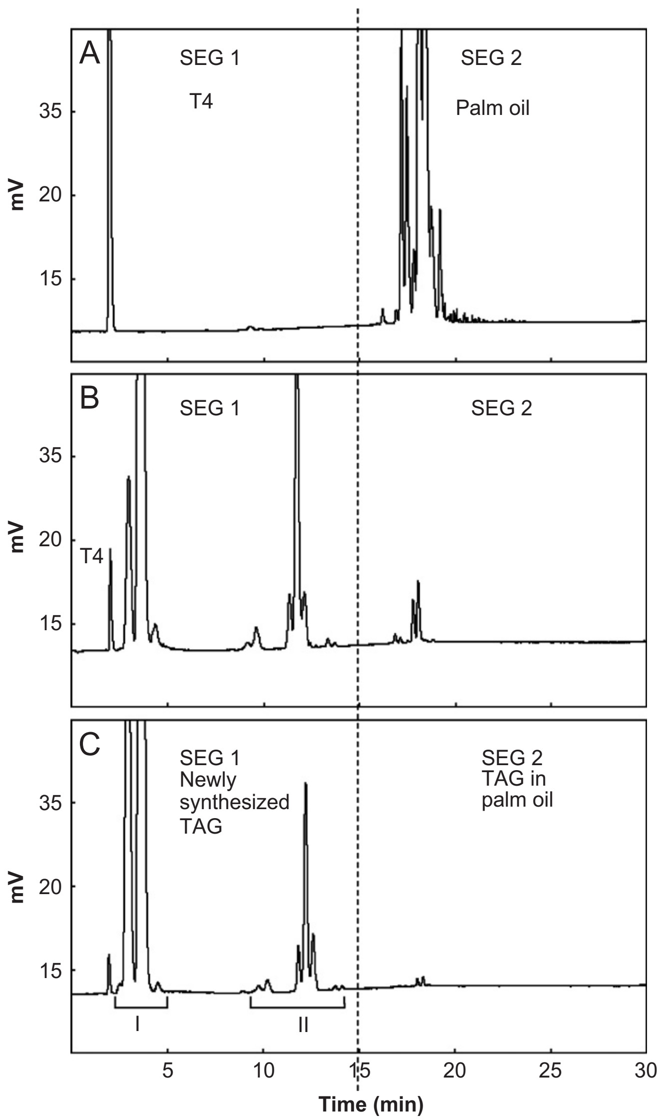

Fig. 3. Reverse-phase HPLC separation of palm oil and tributyrin after 0 h (A), 3 h (B), and 24 h reaction (C).

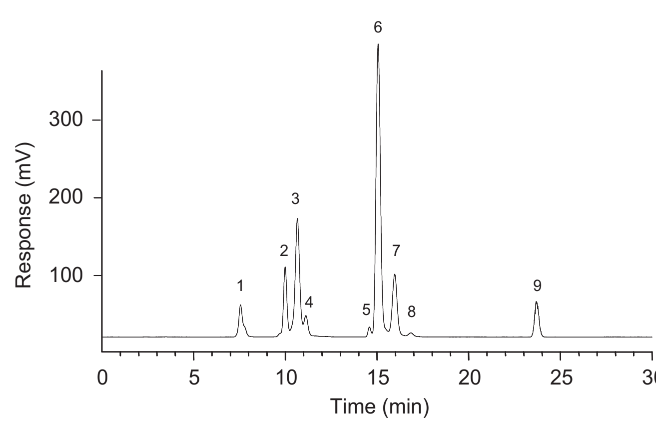

Fig. 4. Chromatogram of the normal-phase HPLC separation of triacylglycerols in the interesterified products from palm oil and tributyrin after 24 h reaction. Designated peak 1: POO, OOO; 2: PBO, OBO, PBP; 3: POB, OOB; 4: OLB, PLB; 5: BBS; 6: PBB, OBB; 7: BPB, BOB, LBB; 8: BLB; and 9: BBB.

Table 2
Mass spectrometric characterization of the proposed triacylglycerol species present in the product (24 h) from interesterified palm oil and tributyrin as separated by normal phase HPLC[a]

Peak number[b]	Retention time (min)	Area%[c]	TAG species[d]	Diacylglycerol fragment ions $[M–RCOO]^{+}$ [e]	Protonated molecules $[M+H]^{+}$ [e]	SD1[f]	SD2[f]	SD3[f]	SD4[f]
1	7.557	4.9	POO, OOO	577, 603	859, 885	OOO			
2	9.997	8.8	PBO, OBO, PBP	383, 409, 551, 577, 603	665, 691	OBO	PBP		
3	10.668	18.9	POB, OOB	409, 577, 603	665, 691	OOB			
4	11.127	3.0	OLB, PLB	383, 407, 409, 575, 601	663, 689				
5	14.582	1.1	BBS	215, 411	ND[g]			BBS	
6	15.064	46.3	PBB, OBB	215, 383, 409	497		PBB		
7	15.968	10.9	BPB, BOB, LBB	215, 383, 407, 409	497	BOB			LBB
8	16.845	0.8	BLB	407	495				BLB
9	23.698	5.3	BBB	215	ND	BBB	BBB	BBB	

[a]Reaction and analysis condition are described in Section 2.
[b]Peak numbers correspond to the normal phase HPLC chromatogram shown in Fig. 4.
[c]Obtained from a Varex MKIII ELSD. Analysis conditions are described in Section 2.
[d]Proposed triacylglycerol (TAG) structure: B, butyryl; P, palmitoyl; O, oleoyl; S, stearoyl; and L, linoleoyl.
[e]Diacylglycerol fragment ions and protonated molecules were detected by HPLC with APCI mass spectrometry. Ions listed in order of detecting intensity.
[f]SD1, reactant of triolein and tributyrin; SD2, reactant of tripalmitin and tributyrin; SD3, reactant of tristearin and tributyrin; and SD4, reactant of trilinolein and tributyrin. Reaction and analysis condition are described in Section 2.
[g]ND, not detected.

To identify the separated peaks, mass spectrometric characterization of each peak from SL-TAG species is proposed in Table 2. Thus, the proposed TAG species identified are listed in Table 2 along with the characteristic diacylglycerol fragment and [M + H] ions that characterized the designated SL-TAG peaks. In addition, the selected standards (tripalmitin, tristearin, triolein, and trilinolein) were interesterified with tributyrin, and their corresponding peaks were compared to those of reaction product based on the retention time. In Table 2 and Fig. 4, SL-TAG species containing one butyryl and two long-chain acyl residues (peaks 2–4) and SL-TAG species containing two butyryl and one long-chain acyl residue (peaks 5–8) were readily separated from TAG composed only of long-chain acyl residues (peak 1) and unreacted tributyrin (peak 9). In mass spectrum, major diacylglycerol fragment ion (m/z; 215) was found in the peaks (peaks 5–7), which is $[M–RCOO]^{+}$ of BB(OH). No $[M–RCOO]^{+}$ with 215 (m/z) was found in peaks 2–4. From Fig. 4, among the SL-TAG species, SL-TAG containing two butyryl and one long-chain acyl residue (e.g., PBB and OBB) were most abundant in this interesterified product, showing 46.3 area% (peak 6). This is followed by SL-TAG containing two long-chain acyl residues and one butyryl (e.g., POB and OOB) at 18.9 area%. Overall, 30.7 area% SL-TAG species containing one butyryl and 59.1 area% SL-TAG species containing two butyryl residues were detected following the reaction conditions in this study. Therefore, among 89.8 area% SL-TAG produced in this study, SL-TAG species containing two butyryl residues dominated when the same weight of both reactants (palm oil and tributyrin) were interesterified. In addition, positional isomers, LBL (e.g., PBO, OBO, and PBP at 9.997 min, peak #2) and LLB/BLL (e.g., POB and OOB at 10.668 min, peak #3) were well separated under our separation condition.

In conclusion, it was found that the NP-HPLC with Chrompack Si60 column readily separated SL-TAG species composed of short- and long-chain acyl residues while the RP-HPLC method was able to separate individual TAG species composed of various acyl moieties. These HPLC methods are effective in analyzing products produced by lipase-catalyzed interesterification and can be used to monitor the content of desired SL-TAG species.

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References

Akoh, C. C. (1995). Structured lipids: enzymatic approach. *INFORM*, *6*, 1055–1061.

Akoh, C. C., & Moussata, C. O. (2001). Characterization and oxidative stability of enzymatically produced fish and canola oil-based structured lipid. *Journal of the American Oil Chemists' Society*, *7*, 25–30.

Basiron, Y. (2007). Palm oil production through sustainable plantations. *European Journal of Lipid Science and Technology*, *109*, 289–295.

Foglia, T. A., Petruso, K., & Feairheller, S. H. (1993). Enzymatic interesterification of tallow–sunflower oil mixtures. *Journal of the American Oil Chemists' Society*, *70*(3), 281–285.

Huang, A. S., Delano, G. M., Pidel, A., Janes, L. E., Softly, B. J., & Templeman, G. J. (1994). Characterization of triacylglycerols in saturated lipid mixtures with application to SALATRIM 23CA. *Journal of Agricultural and Food Chemistry*, *42*(2), 453–460.

Iwasaki, Y., & Yamane, T. (2000). Enzymatic synthesis of structured lipid. *Journal of Molecular Catalysis B: Enzymatic*, *10*, 129–140.

Lee, J. H., Jones, K. C., Lee, K. T., Kim, M. R., & Foglia, T. A. (2003). High-performance liquid chromatographic separation of structured lipids produced by interesterification of Macadamia oil with tributyrin and tricaprylin. *Chromatographia*, *58*(9), 653–658.

Lee, K. T., & Akoh, C. C. (1998). Structured lipids: Synthesis and applications. *Food Reviews International*, *14*, 17–34.

Lee, K. T., Jones, KC., & Foglia, T. A. (2002). Separation of structured lipids by high performance liquid chromatography. *Chromatographia*, *55*(3), 197–202.

Mangos, T. J., Jones, K. C., & Foglia, T. A. (1999). Lipase-catalyzed synthesis of structured low-calorie triacylglycerols. *Journal of the American Oil Chemists' Society*, *76*(10), 1127–1132.

Ruiz-Gutiérrez, V., & Barron, L. J. R. (1995). Review: Methods for the analysis of triacylglycerols. *Journal of Chromatography B*, *671*, 133–168.

Senanayake, S. P. J. N., & Shahidi, F. (2002). Chemical and stability characteristics of structured lipids from borage (*Borago officinalis* L.) and evening primrose (*Oenothera biennis* L.). *Journal of Food Science*, *67*, 2038–2045.

Smith, R. E., Finley, J. W., & Leveille, G. A. (1994). Overview of SALATRIM, a family of low-calorie fats. *Journal of Agricultural Food Chemistry*, *42*, 432–434.

Softly, B. J., Huang, A. S., Finley, J. W., Petersheim, M., Yarger, R. G., Chrysam, M. M., et al. (1994). Composition of representative SALATRIM fat preparations. *Journal of Agricultural Food Chemistry*, *42*, 461–467.

Torres, C. F., Munir, F., Blanco, R. M., Otero, C., & Hill, C. G., Jr. (2002). Catalytic transeseterification of corn oil and tristearin using immobilized lipase form *Thermomyces lanuginose*. *Journal of the American Oil Chemists' Society*, *79*, 775–781.

Willis, W. M., Lencki, R. W., & Marangoni, A. G. (1998). Lipid modification strategies in the production of nutritionally functional fats and oils. *Critical Reviews in Food Science and Nutrition*, *38*, 639–679.